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Nineteen novel *NPHS1* mutations in a worldwide cohort of patients with congenital nephrotic syndrome (CNS)

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Abstract

Background. Recessive mutations in the *NPHS1* gene encoding nephrin account for ~40% of infants with congenital nephrotic syndrome (CNS). CNS is defined as steroid-resistant nephrotic syndrome (SRNS) within the first 90 days of life. Currently, more than 119 different mutations of *NPHS1* have been published affecting most exons.

Methods. We here performed mutational analysis of *NPHS1* in a worldwide cohort of 67 children from 62 different families with CNS.

Results. We found bi-allelic mutations in 36 of the 62 families (58%) confirming in a worldwide cohort that about one-half of CNS is caused by *NPHS1* mutations. In 26 families, mutations were homozygous, and in 10, they were compound heterozygous. In an additional nine patients from eight families, only one heterozygous mutation was detected. We detected 37 different mutations. Nineteen of the 37 were novel mutations (~51.4%), including 11 missense mutations, 4 splice-site mutations, 3 nonsense mutations and 1 small deletion. In an additional patient with later manifestation, we discovered two further novel mutations, including the first one affecting a glycosylation site of nephrin.

Conclusions. Our data hereby expand the spectrum of known mutations by 17.6%. Surprisingly, out of the two siblings with the homozygous novel mutation L587R in *NPHS1*, only one developed nephrotic syndrome before the age of 90 days, while the other one did not manifest until the age of 2 years. Both siblings also unexpectedly experienced an episode of partial remission upon steroid treatment.

Keywords: mutation analysis; nephrotic syndrome; *NPHS1*

Introduction

The protein nephrin [1] is an essential component of the renal glomerular slit diaphragm [2], which is formed by adjacent glomerular epithelial cells (podocytes). The zipper-like structure of the glomerular slit membrane consists of complexes that contain the molecules neph1 and nephrin, which interact between neighbouring podocyte foot processes [3]. Nephrin contains eight immunoglobulin-like domains, a fibronectin type III-like domain, a transmembranous domain and a short intracellular domain [1]

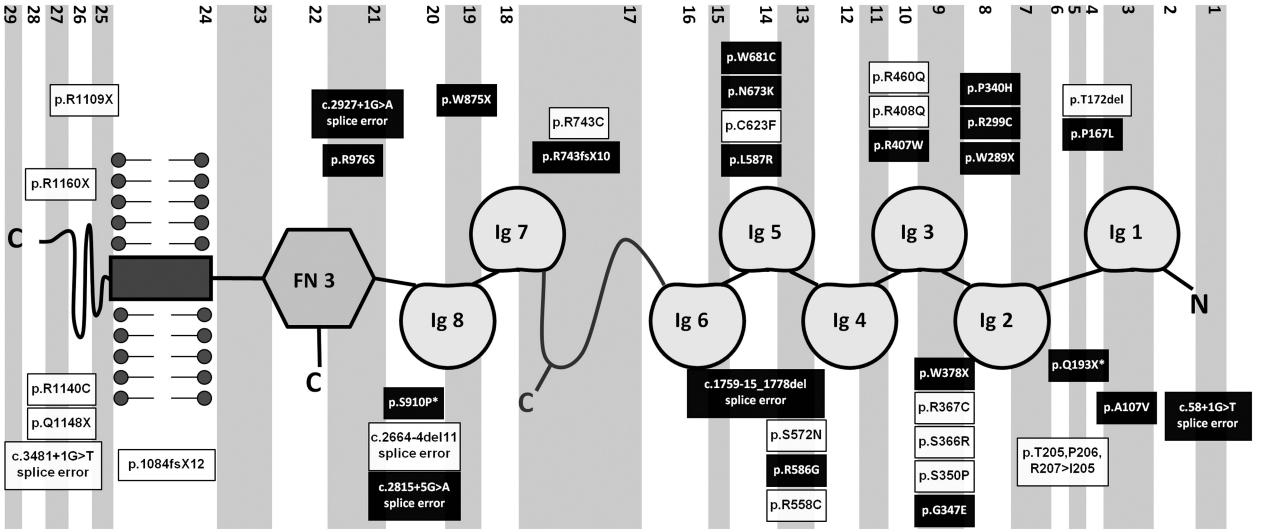

Fig. 1. Localization of mutations in nephrin. The nephrin protein consists of eight extracellular Ig-like domains (Ig 1–8), a fibronectin type III-like module (Ig, FN3), a transmembrane domain (Ig) and a C-terminal (C) cytoplasmic domain (curled line). The grey/white background delimits the exons coding for the corresponding protein domains. All mutations found in this study are listed (novel mutations—white on black; known mutations—black on white). Note that mutations were spread throughout the protein with predominance of Ig-like domain 5. The patient harbouring these mutations was not included in the study cohort (asterisk).

(Figure 1). It plays a significant role in signalling between podocytes by interacting with molecules like CD2AP and podocin [3]. Phosphorylated nephrin binds to Nck, an adapter protein, hereby reorganizing the cell's actin filament network [4]. Recently, an interaction of the intracellular domain of nephrin with β-arrestin was shown to attenuate nephrin signalling [5].

Congenital nephrotic syndrome (CNS) is defined as nephrotic syndrome with onset before the 90th day of postnatal life [6]. Recessive mutations of the nephrin encoding gene *NPHS1* were initially described in the renal histopathological entity of nephrotic syndrome of the 'Finnish type' (CNF) [1]. However, they have more recently also been found outside Finland [7]. Recently, mutations in nephrin were shown to cause ~40% of all cases of CNS [6].

The disease is characterized by massive proteinuria caused by a disruption of the filtration barrier [8]. Due to the massive protein loss, patients often require central venous albumin replacement as well as parental nutrition, leading to a high mortality from septicaemia. End-stage kidney disease (ESKD) before the age of 2–3 years and resistance to standard steroid treatment are the rules. To avoid infectious, thromboembolic and other complications from massive loss of protein, including immunoglobulins and coagulation factors, bilateral nephrectomy, dialysis and renal transplantation at a body weight of 10 kg are recommended [9].

'Congenital nephrotic syndrome of the Finnish type' (CNF) [10,11] is exclusively caused by mutations in *NPHS1* [1]. Renal histology shows microcystic dilatation of the proximal tubules and a progressive mesangial sclerosis [12]. Very recently, rare cases with a manifestation beyond the age of 90 days have also been published, indicating that different mutations in *NPHS1* might cause a spectrum of clinical severity [13].

To date, 119 different mutations in *NPHS1* are known. To expand the spectrum of known mutations, we performed mutational analysis of *NPHS1* by direct sequencing of all exons in 67 patients from 62 different families with CNS.

Materials and methods

Patients and data recruitment

DNA samples and clinical data of a worldwide cohort of 2 056 children with nephrotic syndrome (NS) were ascertained between 1996 and 2008. The diagnosis was made by paediatric nephrologists on the basis of published criteria [14]. Nephrotic-range proteinuria was defined as proteinuria >40 mg/m^2/h. After informed consent was obtained, detailed clinical data and pedigree information were referred to us by the specialists through a standardized clinical questionnaire (www.renalgenes.org) [15]. For all the patients, we performed mutational analysis of *NPHS2* encoding podocin and *WT1*, the most frequent monogenic causes of childhood NS. Human subject research was approved by the University of Michigan Institutional Review Board and the Ethics Commission of the University of Freiburg, Germany. Out of this worldwide cohort, we selected 65 children from 62 different families who had CNS and in whom *NPHS2* (podocin) and *WT1* were excluded. Two patients manifested later, but their siblings had CNS. Also, mutation analysis in phospholipase C epsilon 1 (*PLCE1*) was negative for the six patients with CNS who had a renal histology of diffuse mesangial sclerosis (DMS) [16]. In all 67 patients, mutation analysis for *NPHS1* was performed by PCR with exon-flanking primers followed by direct sequencing. When evaluating frequency of mutations, we relate them to families rather than patients because siblings have identical mutations. When evaluating clinical data, we relate them to patients because siblings might differ in clinical phenotype.

Mutation analysis

Genomic DNA was isolated from blood samples using the Puregene® DNA purification kit (Gentra, Minneapolis, MN) following the manufacturer's guidelines. Mutation analysis was performed by direct sequencing of all 29 exons of *NPHS1*, all eight exons of *NPHS2* and exons 8 and 9 of *WT1*. *WT1* analysis was limited to exons 8 and 9 because mutations of this gene accounting for isolated NS have only been reported in these two exons [17,18]. Additionally, for seven patients with a renal histology of DMS, all exons of *PLCE1* were examined by direct sequencing. Exon-flanking primers for *NPHS1*, *PLCE1*, *NPHS2* and *WT1* have been published previously [15,16,18,19]. For sequence analysis, the software Sequencher 3.8 (Gene Codes, Ann Arbor, MI) was used. As reference for *NPHS1*, the published wild-type sequence (NM_004646) was used for nucleotide and amino acid

Table 1. All *NPHS1* mutations detected

Patient number	Exon	Nucleotide exchange[a]	Effect on coding sequence	Mutation status[b]	Segregation	Reference
Homozygous m*utations*						
A2471	4	c.C500T	p.P167L	Hom	p,m	ps
A2201	4	c.514_516delACC	p.T172del	Hom	nd	Lenkkeri et al. 1999
A3023	8	c.C896T	p.R299C	Hom	nd	ps
A2911	9	c.C1019A	p.P340H	Hom	nd	ps
A1176	9	c.G1040A	p.G347E	Hom	nd	ps
A2031	9	c.A1096C	p.S366R	Hom	nd	Lenkkeri et al. 1999
A2617	9	c.C1099T	p.R367C	Hom	nd	Lenkkeri et al. 1999
CNS11 II-1	9	c.G1134A	p.W378X	Hom	?	ps
CNS11 II-2	9	c.G1134A	p.W378X	Hom	?	ps
A2300	10	c.C1219T	p.R407W	Hom	p,m	ps
A1804	11	c.G1379A	p.R460Q	Hom	nd	Beltcheva et al. 2001
CNS09 II-1	13	c.C1672T	p.R558C	Hom	?	Beltcheva et al. 2001
CNS09 II-2	13	c.C1672T	p.R558C	Hom	?	Beltcheva et al. 2001
A2417	13	c.A1757G	p.R586G	Hom	p,m	ps
A945	14	c.1759-15_1778del	Splice error	Hom	p,m	ps
A2236 II-1	14	c.T1760G	p.L587R	Hom	p,m	ps
A2236 II-2	14	c.T1760G	p.L587R	Hom	p,m	ps
A2355	14	c.T1760G	p.L587R	Hom	p,m	ps
CNS05	14	c.G1868T	p.C623F	Hom	?	Lenkkeri et al. 1999
A2330	20	c.2815+5G>A	Splice error	Hom	p,m	ps
F1273	20	c.2664-4_2670del	Splice error	Hom	nd	Heeringa et al. 2008
CNS03	21	c.2927+1G>A	Splice error	Hom	?	ps
A2088	24	c.3243_3250insG	p.1084fsX12	Hom	nd	Kestila et al. 1998
A2538	24	c.3243_3250insG	p.1084fsX12	Hom	nd	Kestila et al. 1998
A3075	24	c.3243_3250insG	p.1084fsX12	Hom	nd	Kestila et al. 1998
A2553	26	c.C3325T	p.R1109X	Hom	nd	Kestila et al. 1998
CNS12	27	c.3481+1G>T	Splice error	Hom	?	Beltcheva et al. 2001
A2210 II-1	28	c.C3478T	p.R1160X	Hom	nd	Lenkkeri et al. 1999
A2210 II-3	28	c.C3478T	p.R1160X	Hom	nd	Lenkkeri et al. 1999
A2036	28	c.C3478T	p.R1160X	Hom	nd	Lenkkeri et al. 1999
Compound heterozygous m*utations*						
A2341	1	c.58+1G>T	Splice error	Cpd het	m	ps
	3	c.C320T	p.A107V		p	ps
A2535[b]	5	c.C574T	p.Q193X	Cpd het	nd	ps
	20	c.T2728C	p.S910P		nd	ps
A1943	6	c.613_620delinsTT	p.T205,P206,R207>I205	Cpd het	nd	Lenkkeri *et al.* 1999
	13	c.G1715A	p.S572N		nd	Gigante et al. 2005
A2616	6	c.613_620delinsTT	p.T205,P206,R207>I205	Cpd het	m	Lenkkeri et al. 1999
	22	c.G2928T	p.R976S		p	ps
CNS01	8	c.G886A	p.W289X	Cpd het	?	ps
	14	c.T1760G	p.L587R		?	ps
CNS08	9	c.T1048C	p.S350P	Cpd het	?	Lenkkeri et al. 1999
	19	c.G2625A	p.W875X		?	ps
A2030	9	c.A1096C	p.S366R	Cpd het	nd	Lenkkeri et al. 1999
	24	c.C3478T	p.R1160X		nd	Lenkkeri et al. 1999
A3083	9	c.C1019A	p.P340H	Cpd het	nd	ps
	24	c.C3478T	p.R1160X		nd	Lenkkeri et al. 1999
A2475A1981	15	c.G2043T	c.W681C	Cpd het	p	ps
	17	c.2227delC	p.R743fsX10		m	ps
	15	c.C2019A	p.N673K	Cpd het	p	ps
	24	c.C3478T	p.R1160X		m	Lenkkeri et al. 1999
A2249	17	c.C2227T	p.R743C	Cpd het	nd	Lenkkeri et al. 1999
	27	c.C3442T	p.Q1148X		nd	Beltcheva et al. 2001
Single mutations only						
A2618	3	c.C320T	c.A107V	het sm	m	ps
A2380	10	c.G1223A	c.R408Q	het sm	nd	Lenkkeri et al. 1999
A1869	11	c.G1379A	c.R460Q	het sm	nd	Beltcheva et al. 2001
A1517	14	c.G1868T	c.C623F	het sm	nd	Lenkkeri et al. 1999
A2250	17	c.C2227T	c.R743C	het sm	nd	Lenkkeri et al. 1999
A2062	19	c.G2625A	c.W875X	het sm	p	ps
A2320 II-1	23	c.C3418T	c.R1140C	het sm	nd	Lenkkeri et al. 1999
A2320 II-2	23	c.C3418T	c.R1140C	het sm	nd	Lenkkeri et al. 1999

As relevant wild-type gene sequence, the published reference sequence of *NPHS1* was used (NM_004646). het, heterozygous; Hom, homozygous mutation; cpd het, compound heterozygous mutation; sm, single mutation only detected; p, paternal; m, maternal; nd, not done; ?, information not available for this patient; ps, novel mutation detected in the present study.

[a]All mutations were absent from 93 healthy controls.

[b]Patient was not included in the study.

numbering. For all detected mutations and variants, both strands were sequenced. Whenever possible, segregation was confirmed by direct sequencing of the parental samples. For novel mutations, their absence from 93 healthy control individuals was confirmed by direct sequencing.

Homozygosity mapping

Genome-wide homozygosity mapping for 12 families with CNS was performed and evaluated as described previously [20]. Single-nucleotide polymorphism (SNP) arrays (GeneChip®) from Affymetrix, Inc. with a resolution of 250K (Human Mapping 250K Styl Array) were used. Samples were processed, hybridized and scanned using the manufacturer's standard methods at the University of Michigan Core Facility (www.michiganmicroarray.com). Using the software Allegro [21] and ALOHOMORA [22], non-parametric likelihood ratio Z-scores (ZLRs) were calculated using one marker every 100 000 markers. Allele frequencies for Caucasians as specified by Affymetrix®, a disease allele frequency of 0.001, and a standard pedigree structure assuming first-cousin marriage for parents of affected individuals were used. ZLRs were calculated under three different conditions, i.e. for minor allele frequencies of >0.2, >0.3 and >0.4, and a non-existent sibling was included to enable non-parametric Allegro runs. If a peak was constantly exceeding the value of 2.0 in two out of the three conditions, we referred to it as a 'consistent ZLR peak' (cZLR) and expected it to harbour the homozygous mutation of the recessive disease gene [20]. The ZLRs were plotted against genetic distance across the entire human genome using the Gnuplot software (http://www.gnuplot.info) (Supplementary Figure 1; see online supplementary material for a colour version of this figure). In this way, the maxima of ZLR scores represent segments of homozygosity by descent.

Results and discussion

Clinical characteristics and ethnicity of patients

In this study, 67 patients (32 females, 34 males and 1 with unknown gender) from 62 different families with CNS were included. All patients were examined for *NPHS1* mutations.

Renal biopsy was performed in 24 patients, showing 10 with a pattern congruent to NS 'Finnish type', 8 patients with DMS, 3 with minimal change nephrotic syndrome (MCNS), 2 with focal segmental glomerulosclerosis (FSGS), 1 with membranoproliferative glomerulonephritis (MPGN) and 1 with end-stage nephrosclerosis (Supplementary Table 2). Altogether, 21 different ethnicities were represented within the cohort; among these, the largest groups were of Turkish (15%), Arabic (15%), European (13%) and Caucasian (9%) descent (Supplementary Table 2). Consanguinity was reported in 20 families (Supplementary Table 1). In three cases, a nephrectomy had been performed, and 10 patients had received a renal transplant (Supplementary Table 2).

Modality of treatment and response

Because traditionally CNS is considered treatment refractory, steroid treatment was only reported as attempted in 14 (20.8%) cases. Eleven of these patients showed no response (steroid-resistant nephrotic syndrome, SRNS), as expected for CNS, while three (A2236 II-1/II-2, A2380) did partially respond. In four cases, cyclosporin A was applied in addition to steroids, while one patient was treated with cyclosporin A only (Supplementary Table 2). In none of these subjects was any response recorded (Supplementary Table 2, Supplementary Table 4).

'Antiproteinuric therapy' with angiotensin-converting enzyme (ACE) inhibitors or indomethacin was attempted in 34 patients. In nine patients, exclusively ACE inhibitors were applied, of which two (22.2%) showed a partial remission (A1804, A2475). Partial remission is hereby understood as the permanent disappearance of oedema, an increase in the serum albumin concentration to >35 g/L and the persistence of proteinuria of >4 mg/m^2/h [23]. In both patients, mutation analysis had revealed disease-causing mutation in *NPHS1*. Surprisingly, A2475 showed compound heterozygosity for a missense mutation and a deletion. A combined therapy with indomethacin and ACE inhibitors was administered to 26 patients of which eight (30%) showed partial remission, while in 17 cases, no effect was observed. Of the eight patients showing a partial remission, a disease-causing mutation in *NPHS1* was found in six patients. Of these six patients, missense mutations were disease causing in five cases. Unexpectedly, however, patient A2201 also showed partial remission, although a homozygous deletion was detected in this patient. He was treated with captopril for 40 months followed by 10 months of treatment with losartan. Indomethacin was applied for 3 months but did not show any benefit. These data show that 'antiproteinuric' therapy has a beneficial effect on 20–30% of patients with CNS and should not only be considered for patients with missense mutations but might also be positive for patients with a more severe type of mutation (Supplementary Table 2, Supplementary Table 4).

For 19 out of the 67 patients, no pharmacological treatment was reported.

NPHS1 mutations

In 67 children from 62 different families, mutation analysis by direct sequencing of all *NPHS1* exons was performed. In 36 families, causative mutations in *NPHS1* were detected on both alleles. We hereby confirm the results of former studies [6,19] showing that approximately one-half of CNS cases are caused by recessive mutations in *NPHS1* (Table 1). In CNS, it has been shown that ~85% of the cases are explained by mutations in four genes. The distribution among these four genes is: *NPHS1* 39.8%, *NPHS2* 39.8%, *WT1* 2.2% and *LAMB2* 4.4% [6]. Twenty-six of the 36 families showed homozygous mutations, and another 10 families had compound heterozygous mutations. In seven families, only one heterozygous *NPHS1* mutation was detected (Table 1). As a reason for this relatively high number of patients with disease-causing mutations, only on one allele, one might speculate that deletions/duplications of whole exons as well as intronic mutations and mutations in the promoter region can explain these cases. A direct sequencing approach might not have been able to detect these mutations.

Out of the 37 different disease-causing *NPHS1* mutations detected, 19 mutations were novel, consisting of 11 missense mutations, 4 splice-site mutations, 3 nonsense mutations and 1 deletion. They were found in exons 1, 3, 4, 8, 9, 10, 13, 14, 15, 17, 19 and 21 (Table 1 and Figure 1, Supplementary Figure 1; see online supplementary material for a colour version of this figure). The 18 previously published mutations consisted of 10 missense mutations, 3 nonsense mutations, 1 insertion, 1 deletion, 2 splice-site mutations

and 1 insertion/deletion. They were found in exons 4, 6, 9, 10, 11, 13, 14, 17, 20, 22, 24, 26 and 27 (Table 1, Figure 1). None of the patients had the Fin_{major} or the Fin_{minor} mutation. The mutations were broadly distributed over the nephrin protein, affecting all domains. The most frequently affected domains were immunoglobulin (Ig)-like domain 5 and Ig-like domain 3 (Figure 1).

Genotype/phenotype correlations

While the CNS classical histology of 'Finnish type' rapidly progresses into ESKD and shows no response to treatment, several cases of patients with *NPHS1* mutations have been reported, whose histological phenotype was not as severe and who sometimes even showed a response to treatment [19,24].

From the 12 patients in our study, in whom renal biopsy was performed and whose disease was explained by two recessive mutations in *NPHS1*, eight patients showed NS 'Finnish type', three showed MCNS, one showed DMS and one showed FSGS (Supplementary Table 3). These data confirm the previous finding that renal pathology does not exclusively appear as NS 'Finnish type' in CNS caused by recessive *NPHS1* mutations. A higher frequency of mutations in a certain ethnicity was not observed.

Out of the seven patients in our cohort with a biopsy of DMS, only one had disease-causing *NPHS1* mutations (A2911 II-1). This patient showed a novel homozygous missense mutation (c.1019C>A, p.P340H) (Table 1). The patient was steroid resistant. Manifestation was at birth, and ESKD developed at the age of 4 months (Supplementary Table 1). Another patient (A3083 II-1) showed the same mutation heterozygously together with a heterozygous known nonsense mutation [c.3478 C>T (h), p.R1160X] (Table 1). He manifested at the age of 2 months and also showed steroid resistance. Biopsy was not performed, and ESKD was not reported by the age of 6 months (Supplementary Table 1). DMS is seen in patients with mutations in *PLCE1*, *WT1* and *LAMB2*. As *WT1* and *PLCE1* yielded no mutations in the remaining DMS patients, we speculate that they may have mutations in *LAMB2* or *PLCE1*.

One patient (A2616 II-1) with a biopsy of FSGS showed compound heterozygosity for a known deletion (c.613_620delinsTT; p.T205, P206, R207>I205) and a novel missense mutation [c.2928G>T (h), p.R977S] (Table 1). The age of disease onset was 2 months, and steroid treatment was not attempted. The patient developed ESKD by the age of 10 years and was transplanted (Supplementary Table 1).

In two patients with a homozygous *NPHS1* mutation (CNS03, CNS11 II-1), a renal biopsy of MCNS was reported. Patient CNS03 II-1 had a truncating mutation [c.1134G>A (H), p.W378X], while patient CNS11 II-1 had a splice-site error (c.2927 +1G>A, splice error) (Table 1). In both cases, no treatment was attempted. The age of onset for patient CNS03 was shortly after birth, and patient CNS11 II-1 manifested later at the age of 4 months. For patient CNS11 II-2, no biopsy was performed; both siblings were treated with steroids for 2 months but did not respond (Supplementary Table 1). Patient CNS03 showed severe progression with nephrectomy at the age of 6 months and histology of glomerulosclerosis as well as tubular atrophy, microcysts and interstitial fibrosis. As patients with an early biopsy often show MCNS but progress rapidly, the aetiopathology of this patient is not surprising. However, patient CNS11 II-1 is reported to be stabile at the age of 2.5 years now and, together with the late onset, is showing a rather unusual course of disease. Both siblings also showed partial remission to treatment with ACE inhibitors and indomethacin.

Although mutations in *NPHS1* were thought to exclusively cause CNF, these results confirm former findings indicating that *NPHS1* mutations can cause a somewhat broader variety of histological phenotypes in nephrotic syndrome. In a recent genetic study of patients with CNS [6], in a total of 21 patients with two *NPHS1* mutations, the histological phenotypes were distributed as follows: 'Finnish type' (14%), MCNS (14%), FSGS (4.6%), DMS (3.6%), mesangial proliferation (9.2%), mesangial sclerosis (3.6%) and no finding (3.6%) [6] (Supplementary Table 3). In 10 patients, no biopsy was performed. These results were also confirmed by another study of children with CNS, showing again 'non-Finnish type' manifestations [19] (Supplementary Table 3).

While the CNS classical histology of 'Finnish type' rapidly progresses into ESKD and shows no response to treatment, several cases of patients with *NPHS1* mutations have been reported, whose histological phenotype was not as severe and who sometimes even showed a response to treatment [19,24]. Recently, Phillipe *et al.* even reported several cases with childhood rather than congenital onset of nephrotic syndrome and confirmed mutations in *NPHS1* [13]. In this study, two siblings were included who had different age of onset. While the male sibling (A2236 II-2) manifested as CNS by the age of 2.5 months, his elder sister (A2236 II-1) stayed healthy until the age of 24 months. Both showed partial remission due to steroid treatment, and in both sibs, a novel homozygous missense mutation (c.1760T>G, p. L587R) was detected. Partial remission is hereby understood as the permanent disappearance of oedema, an increase in the serum albumin concentration to >35 g/L and the persistence of proteinuria of $>4\,mg/m^2/h$ [23]. A third patient (A2355 II-1), who was classified as CNS and of the same ethnicity, showed the same mutation. Interestingly, he also was not diagnosed before the age of 5 months. Steroid treatment for this patient was not attempted. Additionally, one patient (CNS01) showed L587R heterozygously in combination with a heterozygous nonsense mutation [c.886G>A (h), W289X]. This patient showed the age of onset of 2 months, and no form of steroid treatment was reported. We therefore conclude that homozygous L587R may be a milder mutation, causing a less severe form of nephrotic syndrome than other *NPHS1* mutations with possible childhood onset later than 90 days of life. It is, to our knowledge, the first homozygous mutation in *NPHS1* to cause childhood onset (in A2236 II-1 and A2355 II-1), and the findings suggest that mutation analysis should also be sought in children who manifest after 90 days of life.

Detection of NPHS1 mutations by total genome homozygosity mapping

Mutations that are homozygous by descent have been described as being frequent (30–80%) in paediatric dis-

eases [20]. This can be mapped by homozygosity mapping. In order to investigate if homozygosity mapping is a useful tool for screening, we performed homozygosity mapping in a subset of this cohort of 12 patients from 12 families with different background (Supplementary Table 1). All of them exhibited homozygous segments by inspection of their homozygosity plots, while only five (A2031 II-1, F1273 II-1, A1804 II-1, A2088 II-1, A2036 II-1) revealed homozygosity at the *NPHS1* locus (Supplementary Figure 2; see online supplementary material for a colour version of this figure). We detected homozygous disease-causing mutations of *NPHS1* in all five patients. Of the remaining seven patients, two (A1981 II-1, A2062 II-1) showed compound heterozygous disease-causing mutations, two (A1517 II-1, A1869 II-1) showed single heterozygous mutations and three (A1970 II-1, A1980 II-1, A2112 II-1) showed no mutations of *NPHS1* (Table 1, Supplementary Table 1).

Mutation in N-glycosylation site

In addition to the results of our systematic mutation screening in a CNS cohort, we report on a single patient with onset of nephrotic syndrome at the age of 9 months who was also found to be mutated in *NPHS1*. The patient was not included in the study, and his clinical data is not shown here. He was treated with steroids but did not respond (SRNS). His renal biopsy showed the histological features of IgM nephropathy.

Mutation analysis of *NPHS1* in patient A2535 revealed two novel heterozygous mutations: c.574C>T; p.Q193X and c.2728T>C; p.S910P (Table 1, Figure 1, not included in the examined cohort). This finding is of interest as, to our knowledge, S910P is the first mutation described, which directly affects one of nephrin's ten known *N*-glycosylation sites [25]. Substitution of the serine residue by proline is predicted to prevent glycosylation at this site. Defects in post-translational modification may lead to decreased stability of impaired interaction with other molecules. Considering the late manifestation of nephrotic syndrome in this patient, we speculate that the mutation S910P has some residual protein function and might be a 'mild' mutation. As it has been described recently, a 'mild' mutation in combination with a 'severe' mutation in *NPHS1* may cause childhood onset of nephrotic syndrome [13].

Adding these mutations, we here report 21 novel mutations, expanding the number of published mutations in *NPHS1* by 17.6%.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

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Long-term follow-up of patients with Bartter syndrome type I and II

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Abstract

Background. Little information is available on a long-term follow-up in Bartter syndrome type I and II.

Methods. Clinical presentation, treatment and long-term follow-up (5.0–21, median 11 years) were evaluated in 15 Italian patients with homozygous ($n = 7$) or compound heterozygous ($n = 8$) mutations in the *SLC12A1* ($n = 10$) or *KCNJ1* ($n = 5$) genes.

Results. Thirteen new mutations were identified. The 15 children were born pre-term with a normal for gestational age body weight. Medical treatment at the last follow-up control included supplementation with potassium in 13, non-steroidal anti-inflammatory agents in 12 and gastroprotective drugs in five patients. At last follow-up, body weight and height were within normal ranges in the patients. Glomerular filtration rate was <90 mL/min/1.73 m^2 in four patients (one of them with a pathologically increased urinary protein excretion). In three patients, abdominal ultrasound detected gallstones. The group of patients with antenatal Bartter syndrome had a lower renin ratio ($P < 0.05$) and a higher standard deviation score (SDS) for height ($P < 0.05$) than a previously studied group of patients with classical Bartter syndrome.

Conclusions. Patients with Bartter syndrome type I and II tend to present a satisfactory prognosis after a median follow-up of more than 10 years. Gallstones might represent a new complication of antenatal Bartter syndrome.

Keywords: Bartter syndrome; cholelithiasis; growth retardation; *KCNJ1* gene; *SLC12A1* gene

Introduction

Bartter syndrome type I (BS I) and type II (BS II) are salt-wasting renal tubular disorders that are clinically characterized by polyhydramnios leading to premature delivery, marked polyuria and a tendency towards nephrocalcinosis [1,2]. Loss-of-function mutations either in the furosemide-sensitive sodium–potassium–chloride cotransporter gene (*SLC12A1*; BS I, OMIM 601678) or in the inwardly rectifying potassium channel ROMK gene (*KCNJ1*; BS II, OMIM 241200) have been identified in the vast majority of patients with this autosomal recessive disorder [3,4]. Mutations in the CLCNKB chloride channel gene (Bartter syndrome type III also defined as classical Bartter syndrome—OMIM 607364) as well as in the BSND gene (Bartter syndrome form associated with sensorineural deafness—Bartter type IV—OMIM 602522) are also sometimes responsible for an identical clinical phenotype but will not be treated in this report.